

## MEDICAL APPLICATION OF OXIDIZED MONOTERPENES

### INVENTORS

José Zayas-Rivera, Ph.D., San Juan, Puerto Rico, and  
Naida Montes-Morales, M.S., San Juan, Puerto Rico.

### FIELD OF INVENTION

The invention relates generally to the treatment of fungal, bacterial, yeast and other growths.

### BACKGROUND OF THE INVENTION

Terpenes are a diverse family of compounds with carbon skeletons composed of five carbon isopentyl (isoprene) units. Terpenes are derived from natural sources such as citrus and pine oils, and are generally considered biodegradable. Common forms of terpenes are d-limonene and  $\alpha$ -pinene. Terpenes are classified according to the number of carbon atoms, in units of ten. A terpene with ten carbon atoms is called a monoterpene, one with twenty carbon atoms is a diterpene, and so on.

Various forms of monoterpenes are shown in Fig. 1. These compounds are commercially available. The monoterpenes perillyl alcohol (POH) and perillyl aldehyde (PCO) are both derived from the d-limonene structure. POH is formed by the oxidization of carbon number seven (7), i.e., substitution of hydrogen by a hydroxyl group. PCO is formed by the further oxidization of that carbon, i.e., further substitution of hydrogen by another bond to oxygen.

Fungi, bacteria, and yeast, each may be found in a wide variety of species. While some species serve beneficial purposes, others can cause infections, illnesses, and diseases in humans. For example, Escherichia Coli, a bacteria, is one of the major causes of urinary tract infections as well as diarrhea, sepsis, and meningitis. Pseudonoma aeruginosa is another well-known pathogen that causes infections in wounds and burns. The various species of bacteria respond differently to different treatments.

In medical applications, a treating physician may test for specific bacteria, fungi or yeast. Once identified, the physician may prescribe treatment specific to the microbe

causing the illness. Nevertheless, certain treatments do not provide such clinical identification of the microbe. For example, over-the-counter drugs administered without the aid of a physician are generally applied without specific knowledge of the infecting microbe. Such applications require a composition that acts against a wide variety of infections. POH provides relatively effective treatment against bacteria and yeast. Its effectiveness against fungi, however, is limited.

### Summary of the Invention

According to one aspect of the invention a formulation of PCO is suitable to inhibit the growth of bacteria, fungi, yeast and other growths. According to a further aspect of the invention, the formulation of PCO includes SDS as an activity enhancer. According to another aspect of the invention, the formulation of PCO includes POH as another active ingredient.

According to another aspect of the invention a composition is suitable for use as a fungicide and a bacteriocide. The composition includes perillyl aldehyde, and the at least one inactive ingredient. The concentration of perillyl aldehyde is sufficient to inhibit the growth of fungi and bacteria.

According to another aspect of the invention a composition is capable of inhibiting the growth of fungus or a bacteria. The composition includes perillyl aldehyde, and the at least one inactive ingredient. The concentration of the at least one inactive ingredient is greater than the concentration of perillyl aldehyde.

According to another aspect of the invention the growth of bacteria and fungi is inhibited by application of a composition. The composition has an active ingredient and an inactive ingredient. The active ingredient is perillyl aldehyde.

According to another aspect of the invention the growth of fungi or bacteria is inhibited by the application of a composition. The composition includes perillyl aldehyde as an active ingredient and another inactive ingredient. The concentration of the inactive ingredient is greater than the concentration of the active ingredient.

According to another aspect of the invention the growth of microbes infecting a host is inhibited by application of PCO and POH in a concentration of the at least 0.5% PCO.

According to another aspect of the invention the growth of bacteria or fungi infecting a host is inhibited by application of PCO and SDS.

Further aspects of the invention will be appreciated with reference to the drawings and detailed description.

### **Brief Description of the Drawing**

Figure 1 is a diagram showing various forms of monoterpenes, which are commercially available.

### **Detailed Description**

According to preferred embodiments of the invention (1) PCO, and (2) PCO and POH are formulated for the treatment of bacteria, fungi, yeast, and other growths. Preferred formulations show effective inhibition of the growth of bacteria, fungi and yeast.

The formulations prepared using (1) PCO, and (2) PCO and POH demonstrated anti-bacterial activity with the agar diffusion-disk technique. This technique is explained in R.K. Dart, *Microbiology for the Analytic Chemist*, The Royal Society of Chemists, 1996, which is incorporated herein by reference. Plating seven (7) different bacterial strains, shows the response to the formulations including either PCO or POH or both. Anti-bacterial activity was detected by showing a distinguished halo around the disk previously wetted with the formulation. Halo formation was measured with a caliper and biological activity was considered according to size. Details of specific applications and formulations are set forth below. The following Table 0.1 summarizes various results:

TABLE 0.1: BACTERIAL STRAINS

Formulation	E.coli	Ps.aeruginosa	B.cepacea	S.typhim	S.aureus	S.epiderm	B.subtilis
Control Water	nh	nh	nh	nh	nh	nh	nh
1% SDS	nh	nh	nh	nh	nh	nh	nh
1% PCO, 0.1% SDS	0.52	2.06	0.66	0.21	0.16	0.27	4.19
2% PCO	0.75	4.81	0.8	nm-	0.86	0.52	nm+
1%PCO, 1%POH	1.45	5.59	0.7	0.92	0.79	0.2	5.66
1% POH, 0.1%SDS	0.34	4.1	0.63	0.37	1.09	nm-	4.03

In the above table, nh indicates no-halo; n/a indicates not applicable; nm- indicates not measurable due to small halo; nm+ indicates not measurable due to total inhibition. SDS indicates Sodium Dodecyl Sulfate.

The most sensitive bacterial strain was B. subtilis, which showed a broad halo, providing almost total inhibition of growth.

The 1% PCO formulation produces an immediate decrease in bacterial and yeast concentration. This response is stronger than that observed for POH. The 0.5% PCO/0.5% POH formulation also produces an immediate decrease in bacterial and yeast concentration. This response is stronger than that observed for either PCO or POH in the same concentration.

Although SDS or propylene glycol generally do not provide activity against bacterial cells, they do enhance the inhibition provided by PCO and POH, each alone or in combination. Nonetheless the use of 0.1% SDS was enough to kill S. aureus.

In addition, disinfectant formulations with propylene glycol or polyethylene glycol (PEG 400) show parallel results, these compounds act as an enhancer and help to keep the PCO and/or POH in solution. These enhancers, however, generally have no effect against bacteria, yeast or fungi. Only the primary action of PCO and/or POH was observed.

In addition, the growth of yeast and fungi (specifically *C.albicans* and *A.niger*) are also inhibited by PCO. Details of specific applications and formulations are set forth below. The following Table 0.2 summarizes the results.

TABLE 0.2: FUNGAL STRAINS

Formulation	<i>C. albicans</i>	<i>A. niger</i>
Control Water	nh	nh
1% SDS	nh	nh
1% PCO, 0.1% SDS	6.73	3.83
2% PCO	nm+	n/a
1% PCO, 1%POH	4.6	n/a
1% POH, 0.1%SDS	0.93	2.19

The inhibition power of the formulations with PCO is more effective than with POH for these two organisms. The effect of PCO formulation lasts longer in fungi and yeast than the effect of POH formulation. Specifically, 96 hours after inhibition there was re-growth around the POH formulation, whereas there is no growth around the PCO formulation in *A.niger*. Further experiments have shown that the formulation using PCO at 0.5% is very active against *A.niger* even after 7 days, whereas considerable growth was observed for the same experiment using the same concentration of POH.

Formulations were further tested against a culture of the fungi *A.niger*. The fungi was exposed to 1%PCO/1% POH, 1%PCO, and 1% POH formulations. All formulations were prepared in a glycerol/ SDS base in water. In this test, after 18 hours of exposure the 1%PCO/1% POH formulation killed the fungi where it was placed and limited the growth over a 2.44 mm halo around the disk moistened with the formulation. The formulations of 1% PCO and 1% POH did not show a measurable halo but did inhibit the growth in the area where it was placed. Furthermore, after 12 days of continued incubation the 1%PCO/1%POH showed no growth in the halo and appears to have a limited growth at the edge of the halo. Moreover, no growth was observed at the top of the disk and no growth beneath the disk. Also no growth was observed in the disk top of the other two formulations. Under more controlled conditions a 0.5 and 1% formulations of PCO did inhibit growth of *A.niger*, whereas POH did not perform as aggressively.

Moreover, *C. albicans* was cultured in Sabouraud-Dextrose media and was exposed to a cream formulation having 0.75% perillaldehyde. A well was made in the middle of the agar and filled with the 0.75% perillaldehyde cream. After 24 hours, the distinguished halo appeared as soon as the diffusion of active ingredient (PCO) passes across the agar.

The following examples illustrate the antibacterial and antifungal properties of various formulations. The formulations are applied to common bacterial fungal and yeast strains.

**Example 1: *E. coli***

Again, *E. coli* is one of the major causes of urinary tract infections as well as diarrhea, sepsis and meningitis. The formulations were exposed against cultures of *E. coli* showing growth inhibition.

About  $1-2 \times 10^6$  cells of *E. coli* were exposed to 1% PCO, 1%PCO/1%POH, 1% POH and 0.5%PCO/0.5%POH formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulations in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance (Abs.) measures were taken at 600nm. After 10 minutes of exposure the bacterial growth was inhibited.

The following tables 1.1, 1.2, 1.3, and 1.4, show the responses for 1% PCO, 1% PCO/1% POH, 1% POH and 0.5% PCO/0.5% POH formulations, respectively.

**Table 1.1: 1% PCO Formulation**

Exposure time	<b>E. coli Abs. 600nm</b>
0 minutes	0.8912
10 minutes	0.0032
30 minutes	0.0033
60 minutes	0.0019

**Table 1.2: 1%PCO/1%POH Formulation**

Exposure time	<b>E. coli Abs. 600nm</b>
0 minutes	0.9254
10 minutes	0.0217
30 minutes	0.0126
60 minutes	0.0609

**Table 1.3: 1% POH Formulation**

<b>Exposure time</b>	<b>E. coli Abs. 600nm</b>
0 minutes	0.8727
10 minutes	0.0057
30 minutes	0.0050
60 minutes	0.0067

**Table 1.4: 0.5% PCO 0.5% POH Formulation**

<b>Exposure time</b>	<b>E. coli Abs. 600nm</b>
0 minutes	0.8628
10 minutes	0.1031
30 minutes	0.1017
60 minutes	0.0812

**Example 2: Pseudomona aeruginosa**

Pseudonoma aeruginosa (P.aeruginosa) is a well known pathogen which is the cause of infections in wounds and burns. It also causes meningitis and urinary tract infections. If it invades the bloodstream system, it may result in fatal sepsis, and skin infections causing necrosis and ecthyma gangrenosum.

About  $1-2 \times 10^6$  cells of P. aeruginosa were exposed to 1% PCO, 1%PCO/1%POH, 1% POH and 0.5%PCO/0.5%POH formulation at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance measures were taken at 600 nm. After 10 minutes of exposure the bacterial growth was inhibited.

The following Tables 2.1, 2.2, 2.3, and 2.4, show the responses for 1% PCO, 1% PCO/1% POH, 1% POH, and 0.5% PCO/0.5% POH formulations, respectively.

**Table 2.1: 1% PCO Formulation**

<b>Exposure time</b>	<b>Ps. Aeruginosa Abs. 600nm</b>
Control	0.2582
10 minutes	0.0039
30 minutes	0.0004
60 minutes	0.0050

**Table 2.2: 1%PCO/1%POH Formulation**

<b>Exposure time</b>	<b>P. aeruginosa Abs. 600nm</b>
Control	0.7948
10 minutes	0.0215
30 minutes	0.0142
60 minutes	0.0429

**Table 2.3: 1%POH Formulation**

<b>Exposure time</b>	<b>P. aeruginosa Abs. 600nm</b>
Control	0.2089
10 minutes	0.0129
30 minutes	0.0200
60 minutes	0.0262

**Table 2.4: Ps. aeruginosa 0.5% PCO/ 0.5% POH Formulation**

<b>Exposure time</b>	<b>P. aeruginosa Abs. 600nm</b>
Control	0.2089
10 minutes	0.0129
30 minutes	0.0200
60 minutes	0.0262

**Example 3: Burkholderia cepacea**

Burkholderia cepacea (B.cepacea) is found in patients with cystic fibrosis, and other pulmonary infections.

About  $1-2 \times 10^6$  cells of B.cepacea were exposed to 1%PCO, 1%POH, 1%PCO/1%POH, and 0.5%PCO/0.5%POH formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance measures were taken at 600 nm. After 10 minutes of exposure the bacterial growth was inhibited. B. cepacea exhibited a low growth of control cells. Nonetheless, the diminishing in the absorbance values and the lack of turbidity in the broth culture shows growth inhibition.

The following tables 3.1, 3.2, 3.3, and 3.4, show the responses for 1% PCO, 1% POH, 1% PCO/1% POH, and 0.5%PCO/0.5% POH, respectively.

**Table 3.1: 1% PCO Formulation**

Exposure time	B. cepacea abs. 600nm
Control	0.1533
10 minutes	0.0110
30 minutes	0.0076
60 minutes	0.0072

**Table 3.2: 1% POH Formulation**

Exposure time	B. cepacea abs. 600nm
Control	0.0286
10 minutes	0.0107
30 minutes	0.0079
60 minutes	0.0101

**Table 3.3: 1% PCO 1% POH Formulation**

Exposure time	B. cepacea absorbance 600 nm
Control	0.1533
10 minutes	0.0686
30 minutes	0.0427
60 minutes	0.0645

**Table 3.4: 0.5% PCO 0.5% POH Formulation**

Exposure time	B. cepacea abs. 600nm
Control	0.1533
10 minutes	0.075
30 minutes	0.0355
60 minutes	0.0557

**Example 4: *Salmonella typhimurum***

Salmonella Typhimurum (*S.typhimurum*) causes typhoid fever or enteric fever and gastroenteritis or enterocolitis. Typhoid fever symptoms are headache, enlargement of the liver and spleen, and rose spots. Other lesions are hyperplasia and necrosis of lymphoid tissue.

About  $1-2 \times 10^6$  cells of *S. typhimurum* were exposed to 1% PCO, 1%POH 1%PCO/1%POH, and 0.5%PCO/0.5%POH formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 –24 hours.

Absorbance measures were taken at 600 nm. After 10 minutes of exposure, the bacterial growth was inhibited.

The following tables 4.1, 4.2, 4.3, and 4.4, show the responses for 1% POC, 1% POH, 1% PCO/1% POH, and 0.5% PCO/0.5% POH formulations, respectively.

**Table 4.1: 1% POC Formulation**

Exposure time	S. typhimurum abs. 600nm
Control	0.8172
10 minutes	0.0053
30 minutes	0.0053
60 minutes	0.0052

**Table 4.2: 1% POH Formulation**

Exposure time	S. typhimurum abs. 600nm
Control	0.8577
10 minutes	0.0237
30 minutes	0.0228
60 minutes	0.0130

**Table 4.3: 1% POH/ 1%PCO Formulation**

Exposure time	S. typhimurum abs. 600nm
Control	0.3128
10 minutes	0.0187
30 minutes	0.0138
60 minutes	0.0695

**Table 4.4: 0.5% PCO, 0.5% POH Formulation**

Exposure time	S. typhimurum abs. 600nm
Control	0.9274
10 minutes	0.1064
30 minutes	0.1231
60 minutes	0.0926

**Example 5: Staphylococcus aureus:**

Staphylococcus aureus (S.aureus) causes Toxic Shock Syndrome, and also causes wound skin infections, bacteremia, endocarditis (caused by contaminated medical devices), meningitis, hematogenous osteomyelitis, or pulmonary infections. Bacteremia is particularly hard to cure because it develops resistance to antibiotics.

About  $1-2 \times 10^6$  cells of *S. aureus* were exposed to 1%PCO, 1%POH, 1%PCO/1%POH, 0.5%PCO/0.5%POH, 0.5% PCO/0.006% SDS, and 0.25% PCO/0.003% SDS formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance measures were taken at 600 nm. In addition, *S. aureus* was susceptible to 0.1% SDS in positive control. Lowering the percentage of SDS (to 0.003% SDS) showed growth in the positive controls. After ten minutes in terpene (mixture of PCO and POH) exposure in formulation low in SDS, the bacterial growth was inhibited.

The following Tables 5.1, 5.2, 5.3, 5.4, 5.5, and 5.6, show the responses for 1% PCO, 1% POH, 1%PCO/1%POH, 0.5% PCO/0.006% SDS, and 0.25% PCO/0.003% SDS, respectively.

**Table 5.1: 1% PCO Formulation**

<b>Exposure time</b>	<b><i>S. aureus</i> abs. 600nm</b>
Control	0.9945
10 minutes	0.0422
30 minutes	0.0464
60 minutes	0.0426

**Table 5.2: 1% POH Formulation**

<b>Exposure time</b>	<b><i>S. aureus</i> abs. 600 nm</b>
Control	0.9945
10 minutes	0.0135
30 minutes	0.0183
60 minutes	0.0111

**Table 5.3: 1% PCO/1% POH Formulation**

<b>Exposure time</b>	<b><i>S. aureus</i> abs. 600 nm</b>
Control	0.9945
10 minutes	0.0200
30 minutes	0.0210
60 minutes	0.0218

**Table 5.4: 1% Terpene Formulation (0.5% PCO/0.5% POH)**

Exposure time	S. aureus absorbance at 600 nm
Control	0.8376
10 minutes	-0.0103
30 minutes	-0.007
60 minutes	-0.0027

**Table 5.5: 0.5% PCO, 0.006% SDS Formulation**

Exposure time	S. aureus absorbance at 600 nm
Control	0.8376
10 minutes	-0.0029
30 minutes	-0.0062
60 minutes	-0.0021

**Table 5.6: 0.25% PCO, 0.003% SDS Formulation**

Exposure time	S. aureus abs. 600nm
Control	0.8376
10 minutes	0.0237
30 minutes	0.0178
60 minutes	0.0186

**Example 6: Staphylococcus epidermidis:**

Staphylococcus epidermidis (S. epidermidis) are part of the normal human flora of the skin and respiratory and gastrointestinal tracts. Nonetheless S. epidermidis can cause infection and become resistant to antibiotics.

About  $1-2 \times 10^6$  cells of S.epidermidis were exposed to 1%PCO, 1%POH, 1%PCO/1%POH, and 0.5%PCO/0.5%POH formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance measures were taken at 600 nm. After 10 minutes of exposure, the bacterial growth was inhibited.

The following Tables 6.1, 6.2, 6.3, and 6.4, show the responses for 1% PCO, 1% POH, 1% PCO/1% POH, and 0.5% PCO/0.5% POH Formulations, respectively.

**Table 6.1: 1% PCO Formulation**

Exposure time	S. epidermidis abs. 600nm
Control	0.3153
10 minutes	0.0418
30 minutes	0.0448
60 minutes	0.0394

**Table 6.2: 1% POH Formulation**

Exposure time	S. epidermidis abs. 600nm
Control	0.3153
10 minutes	0.0119
30 minutes	0.0136
60 minutes	0.0162

**Table 6.3: 1% PCO/1%POH Formulation**

Exposure time	S. epidermidis abs. 600nm
Control	0.3153
10 minutes	0.0283
30 minutes	0.0232
60 minutes	0.0210

**Table 6.4: 0.5% PCO, 0.5% POH Formulation**

Exposure time	S. epidermidis abs. 600nm
Control	0.1764
10 minutes	0.0447
30 minutes	0.0111
60 minutes	0.0006

**Example 7: *Bacillus subtilis*:**

*Bacillus subtilis* (*B. subtilis*) is not a normal flora member, but is not a pathogen either. It is produced by spores, and is one of the control organism used in autoclave effectiveness.

About  $1-2 \times 10^6$  cells of *B. subtilis* were exposed to 1% PCO, 1% POH, 1% PCO/1%POH and 0.5% PCO/0.5% POH formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance measures were taken at 600 nm. After 10 minutes of exposure the bacterial growth was inhibited.

The following Tables 7.1, 7.2, 7.3, and 7.4, show the responses to 1% PCO, 1% POH, 1% PCO/1%POH and 0.5% PCO/0.5% POH formulations, respectively.

**Table 7.1: 1% PCO Formulation**

<b>Exposure time</b>	<b>B. subtilis abs. 600nm</b>
Control	0.9077
10 minutes	0.0006
30 minutes	-0.0058
60 minutes	-0.0113

**Table 7.2: 1% POH Formulation 1%**

<b>Exposure time</b>	<b>B. subtilis abs. 600nm</b>
Control	0.9077
10 minutes	0.0248
30 minutes	0.0269
60 minutes	0.0463

**Table 7.3: 1% PCO/1% POH Formulation**

<b>Exposure time</b>	<b>B. subtilis abs. 600nm</b>
Control	0.9077
10 minutes	0.0165
30 minutes	0.0300
60 minutes	0.0205

**Table 7.4: 0.5% PCO, 0.5% POH Formulation**

<b>Exposure time</b>	<b>B. subtilis abs. 600nm</b>
Control	1.1278
10 minutes	0.0351
30 minutes	0.0616
60 minutes	0.0570

**Example 8: *Candida albicans*:**

*Candida albicans* (*C. albicans*) causes vulvovaginal infections. Usually irritation, pruritus, and vaginal discharge are present. This is often lead by factors such as diabetes, pregnancy and some antibacterial drugs that alter the normal flora.

About  $1-2 \times 10^6$  cells of *C.albicans* were exposed to 1%PCO, 1%POH, 1%PCO/1%POH, and 0.5% PCO/0.5% POH, formulations at 10, 30, and 60 minutes. Once the fungi were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition

mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 18 – 24 hours. Absorbance measures were taken at 600 nm. After 10 minutes of exposure, the fungal growth was inhibited.

The following Tables 8.1, 8.2, 8.3, 8.4, show the responses to 1% PCO, 1% POH, 1% PCO/1% POH, and 0.5% PCO/0.5% POH formulations, respectively.

**Table 8.1: 1% PCO FormULATION**

Exposure time	C. albicans abs. 600nm
Control	0.4093
10 minutes	0.0050
30 minutes	0.0070
60 minutes	0.0054

**Table 8.2: 1% POH FORMULATION**

Exposure time	C. albicans abs. 600nm
Control	0.4093
10 minutes	0.0529
30 minutes	0.0332
60 minutes	0.0449

**Table 8.3: 1% PCO/1% POH FORMULATION**

Exposure time	C. albicans abs. 600nm
Control	0.4093
10 minutes	0.0083
30 minutes	0.0240
60 minutes	0.0225

**Table 8.4: 0.5% PCO/0.5% POH FORMULATION**

Exposure time	C. albicans abs. 600nm
Control	0.2067
10 minutes	0.0350
30 minutes	0.0227
60 minutes	0.0350

#### **Example 9: Aspergillus niger**

Aspergillus niger (A. niger) causes aspergillosis, especially in immunosuppressive patients. Non-invasive infections may involve the ear canal, cornea or the nails. In invasive aspergillosis the symptoms may include fever, cough, dyspnea, hemoptysis, or may cause thrombosis, infarction and necrosis.

About  $1 \times 10^6$  cells of *Aspergillus niger* were exposed to 1% PCO, 1% POH and 0.5%PCO/0.5%POH formulations at 10, 30, and 60 minutes. Once the bacteria were exposed to formulation in the determined time, a 0.5 ml aliquot of exposition mix was used to inoculate 10 ml of TSB media and incubated at 35°C for 48 hours. Absorbance measures were taken at 600 nm. After 10 minutes of exposure, the fungal growth was inhibited.

The following Tables 9.1, 9.2, and 9.3, show the responses to 1% PCO, 1% POH and 1% Terpene (0.5%PCO/0.5%POH) formulations, respectively.

**Table 9.1: 1% PCO Formulation**

<b>Exposure time</b>	<b>A. niger Abs. 600nm</b>
Control	0.4394
10 minutes	0.0137
30 minutes	0.0149
60 minutes	0.0163

**Table 9.2: 1% POH Formulation**

<b>Exposure time</b>	<b>A. niger Abs. 600nm</b>
Control	0.4394
10 minutes	0.0316
30 minutes	0.0204
60 minutes	0.0092

**Table 9.3: 1% Terpene (0.5% PCO/0.5%POH) Formulation**

<b>Exposure time</b>	<b>A. niger Abs. 600nm</b>
Control	0.4394
10 minutes	0.0411
30 minutes	0.0459
60 minutes	0.0326

Turning now to preferred formulations, a cream, an ointment, a gel, and a dry and wet formulation are described.

A preferred cream formulation includes PCO as an active ingredient, and octadecanol, propanediol, hexadecanol, oleic acid, and mineral oil, as inactive ingredients. The cream formulation acts as a bactericide, fungicide and disinfectant. The cream formulation is

suitable to treat infections such as skin (pruritus) or vaginal candidiasis, skin wounds, and burns. It is also suitable to treat against nail or ear aspergillosis and other skin fungi.

Table 10.1 below lists the specific composition of a preferred 1% PCO formulation.

Order	Reagent	Amount ml	Amount	%
1	Octadecanol	--	1.0g	14.091
2	Propanediol	5 ml	5.18g	72.994
3	Hexadecanol	--	0.8g	11.273
4	Oleic acid	0.025 ml	0.022275g	0.314
5	Mineral oil	0.025 ml.	0.021875g	0.308
6	PCO	0.075 ml	0.0723375g	1.019
Total		7.0964875g	100.00%	

Table 10.2 below lists the specific composition of a 0.76% PCO formulation.

Order	Reagent	Amount ml	Amount	%
1	Octadecanol	--	1.0g	10.5
2	Hexadecanol	--	0.8g	8.4
3	Propanediol	7.0 ml	7.252g	76.16
4	Oleic acid	0.025 ml	0.022275g	0.23
5	Mineral oil	0.200 ml	0.175g	1.84
6	DI Water	0.200 ml	0.2g	2.1
7	PCO	0.075 ml	0.072338g	0.76
Total		9.521613	100.00%	

A preferred ointment formulation includes PCO as an active ingredient and bees wax, mineral oil, and hexadecanol, as inactive ingredients. The ointment formulation is suitable to treat infections such as skin (pruritus) or vaginal candidiasis, skin wounds and burns, against nail or ear aspergillosis and other skin fungi.

Table 11.1 below lists the specific composition of a preferred 1% PCO ointment.

Order	Reagent	Amount ml	Amount	%
1	Bees Wax	--	1.2g	11.68
2	Mineral Oil	10 ml	8.75g	85.179
3	Hexadecanol	--	0.2g	1.947
4	PCO	0.127 ml	0.1225g	1.193
	Total		7.0964875g	100.00%

A preferred gel includes PCO as an active ingredient, and hydroxypropyl cellulose, Tween 60, DI water, carbopol 940, sodium bicarbonate and isopropanol 70% as inactive ingredients. The gel formulation is suitable to treat infections such as skin (pruritus) or vaginal candidiasis, skin wounds and to prevent burn infections, against nail or ear aspergillosis and other skin fungi.

Table 12.1 below lists the specific composition of one preferred gel formulation.

#### Gel Formulation – No PCO

Order	Reagent	Amount ml	Amount	%
1	Hydroxypropyl cellulose	-	0.6 g	0.545
2	Tween 60	-	4.0 g	3.635
3	DI Water	100.0 ml	100.0 g	90.884
4	Carbopol 940	-	1.0 g	0.908
5	Sodium Bicarbonate	-	0.5 g	0.454
6	Isopropanol 70 %	5.0 ml	3.93 g	3.572
	Total		110.03 g	99.998%

25g of Gel Formulation were used and 0.1206g of PCO was added for a 0.5% PCO gel. Table 12.2 below lists the specific composition of one preferred 0.5% PCO gel formulation.

Order	Reagent	Amount g	%
1	Hydroxypropyl cellulose	0.1363g	0.543
2	Tween 60	0.9088	3.618
3	DI Water	22.7211	90.448
4	Carbopol 940	0.2272	0.904
5	Sodium Bicarbonate	0.1136	0.452
6	Isopropanol 70%	0.8929	3.554
7	Perillyl aldehyde	0.1206g	0.480
Total		25.1205	99.999

A preferred dry formulation includes PCO as an active ingredient and SDS as an enhancer. The dry formulation is suitable to be packed in pouches such as Hydrolene® material for household, pharmaceutical or hospital critical cleaning and disinfection, and germ-free prosthetic devices by dilution directly into water. This avoids bulky bottles for packaging and allows shipment of low weight pouches instead of, for example, one-gallon bottles.

One preferred formulation having 22.5% PCO stock includes 1 ml PCO (90%), 1 ml 2.5% SDS in propylene glycol, and 2 ml propylene glycol (or polyethylene glycol).

Another preferred formulation having 21.25% POH stock includes 1 ml POH 85%, 1 ml 10% SDS in propylene glycol, and 2 ml propylene glycol.

Another preferred formulation having 11.9% PCO and 11.9% POH includes 0.55 ml PCO (90%), 0.58 ml POH, 1 ml 10% SDS in propylene glycol, and 2 ml propylene glycol (or polyethylene glycol).

Preferred Wet Formulations are suitable for household, pharmaceutical or hospital critical cleaning and disinfection limiting the propagation of gastroenteritis, enterocolitis, pneumonia, and other nosocomials diseases. In addition, these formulations are useful for disinfections of prosthetic devices, surgical clothes disinfection, or in wastewater treatment

plants, as well as in meat or poultry food manufacturing plants. These formulations are also suitable in atomizer bottle sprays over bookshelves against books mold and fungi.

One preferred formulation having a 1% PCO solution includes 1 ml PCO stock, and 21.5 ml deionized ultra filtered water.

Another preferred formulation having a 1% POH solution includes 1 ml of POH stock, and 20.25 ml of deionized ultra filtered water.

Yet another preferred formulation having a 1% terpene solution includes 1 ml of terpene stock, and 22.8 ml of deionized ultra filtered water.

Yet another preferred formulation having a 50% terpene solution includes 1.11 ml of 90% PCO, 1.17 ml of 85% POH, 1 ml of 10% SDS in propylene glycol, and 2 ml of propylene glycol.

Yet another preferred formulation having a 2% terpene solution includes 1 ml of 50% Terpene stock, 24 ml of deionized ultra filtered water.

Although the invention has been described with reference to specific preferred applications and formulations, those skilled in the art will appreciate that many modifications and variations to such applications and formulations may be made without departing from the teachings of the invention. All such modifications and variations are intended to be encompassed within the scope of the following claims.